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Cognitive impairments are different in single-incidence and multi-incidence ADHD families

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Conflict of interest

J.K. Buitelaar has been a consultant to/member of advisory board of/and/or speaker for Janssen Cilag BV, Eli Lilly, Bristol-Myer Squibb, Organon/Shering Plough, UCB, Shire, Medice, Roche and Servier. B. Franke is supported by a Vici grant from the Netherlands Organization for Scientific Research (NWO grant # 016.130.229). All other authors report no biomedical financial interests or potential conflicts of interest.

Abstract

Background: We may improve our understanding of the role of common versus unique risk factors in attention-deficit/hyperactivity disorder (ADHD) by examining ADHD-related cognitive deficits in single- (SPX), and multi-incidence (MPX) families. Given that individuals from MPX families are likely to share genetic vulnerability for the disorder, whereas SPX ADHD may be the result of sporadic (non-)genetic causes unique to the patient, we hypothesized that cognitive impairments may be different in SPX and MPX ADHD as indicated by (a) the presence of cognitive deficits in MPX, but not SPX unaffected siblings and (b) dissimilar cognitive profiles in SPX and MPX ADHD patients.

Methods: Tasks measuring total IQ, verbal attention, executive functioning, motor functioning, and time estimation were administered to 31 SPX/264 MPX ADHD probands, 47 SPX/123 MPX unaffected siblings, and 263 controls, aged 6-19 years.

Results: SPX unaffected siblings were unimpaired compared to controls, except for verbal working memory, whereas MPX unaffected siblings showed impairments on most cognitive domains. The cognitive profiles of SPX and MPX probands were highly similar, except that verbal attention, response inhibition and motor control deficits were more pronounced in MPX probands, and -compared to their unaffected siblings- impairments in IQ, visual working memory and timing abilities were more pronounced in SPX cases.

Conclusions: Our results support the hypothesis that a partly different cognitive architecture may underlie SPX and MPX forms of ADHD, which becomes evident when contrasting cognitive performances *within* families. Cognitive factors underlying MPX forms of ADHD are familial, whereas non-familial in SPX ADHD. SPX-MPX stratification may be a step forward in unravelling diverse causal pathways.

Keywords: Attention-Deficit/Hyperactivity Disorder (ADHD); simplex-multiplex stratification; family; unaffected relative; endophenotype

Abbreviations:

ADHD = attention-deficit/hyperactivity disorder

SPX = simplex

MPX = multiplex

ASD = autism spectrum disorders

SSRT = stop signal reaction time

LMM = linear mixed models

Attention-deficit/hyperactivity disorder (ADHD) is a severely impairing neurodevelopmental disorder, characterized by symptoms of hyperactivity, impulsivity and/or inattention (Diagnostic and Statistical Manual of Mental Disorders; DSM-5) (APA, 2013). ADHD is a highly heritable disorder, with heritability estimates ranging to 76% (Faraone et al., 2005, Thapar et al., 2013). Common to ADHD is the large within-disorder heterogeneity, in symptom presentation, developmental course and underlying etiological mechanisms (Wahlstedt et al., 2009). The prevailing etiological model suggests that ADHD is caused by small disease-increasing effects of multiple common genetic and environmental risk factors (Franke et al., 2009, Thapar et al., 2013). However several recent studies report that rare genetic mutations or non-shared environmental factors (such as low birth weight and medical conditions) with a large effect may relate to ADHD aetiology as well (Williams et al., 2010, Ben Amor et al., 2005). This suggests that while in many cases multifactorial factors, possibly shared with (unaffected) relatives, might underlie ADHD, factors uniquely present in affected individuals, such as *de novo* mutations, might underlie the disorder in at least some cases.

More insight into the role of shared versus unique genetic factors for ADHD might be obtained by examining the presence of ADHD-related cognitive deficits in unaffected siblings of ADHD probands in the search for cognitive endophenotypes of ADHD. Endophenotypes are defined as heritable vulnerability traits that heighten the risk for developing a disorder (Gottesman and Gould, 2003). Endophenotypes offer a simplified approach to dissect complex traits by reducing heterogeneity and as such may boost the power for genetic analyses, as well as shed light on the functional outcomes of genes (Gottesman and Gould, 2003). Cognitive deficits that are present in unaffected siblings and thus shared between affected and unaffected relatives are assumed to provide an index of the multifactorial liability to ADHD (Waldman, 2005). Conversely, cognitive deficits that are not shared between affected and unaffected siblings may have a unique effect on the development of the disorder. This affected-unaffected siblings design has been frequently applied in ADHD research and has led to many studies documenting an increased incidence of behavioral symptoms, comorbid symptomatology, and ADHD-related cognitive deficits in unaffected family members of ADHD probands (for an extensive review see Rommelse et al., 2011).

We may improve our understanding of the role of common versus unique genetic risk factors in ADHD by examining ADHD related cognitive deficits in single-, and multi-incidence ADHD families. We hypothesized that ADHD-related cognitive deficits are only present in multi-incidence (here referred to as multiplex; MPX), but not single-incidence (here referred to as simplex: SPX) ADHD families. SPX families are defined as nuclear families with only one affected individual and at least one unaffected male sibling. MPX families consist of at

least two (or more) affected individuals in the family (Sullivan et al., 2012). The assumption is that individuals from SPX families are more likely than individuals from MPX families to develop ADHD as a result of sporadic genetic and/or non-genetic causes strictly unique to the patient. Then unaffected relatives in SPX families would show less or even no behavioral or cognitive deficits compared to controls and would deviate more from the cognitive profile of their affected brother or sister. In contrast, unaffected relatives in MPX families would show cognitive deficits, compared to controls, and about as similar as the probands. In other words, the within-family contrast between probands and unaffected siblings regarding cognitive or behavioral aspects of the disorder is larger in SPX compared to MPX families. Unaffected siblings can be viewed as an ideal reference group, indexing the ‘full potential’ of children with ADHD had they not developed the disorder (while correcting for shared environmental influences). Higher within-family contrasts might thus be indicative of more severely impaired cognitive abilities in the affected children from those families. This model of different etiologies in SPX and MPX families has been developed and confirmed in research in Autism Spectrum Disorders (ASD) (Gerdtts et al., 2013, Sebat et al., 2007). For example, a more than threefold rate of de novo mutations were identified in ASD SPX families (~7-10%), compared to ASD MPX families (~2-3%) or control families (~1%) (Sebat et al., 2007). In contrast, members of MPX families more often exhibit ASD traits compared to members of SPX families, indicative of a more pronounced role of shared genetic predispositions (Gerdtts et al., 2013). The association between de novo mutations and ADHD has received little research attention, unlike ASD (D’Onofrio et al., 2014). Recent studies that point towards a role for rare genetic variants such as de novo mutations in ADHD highlight the need for future studies exploring this issue (Williams et al., 2010, Ben Amor et al., 2005, D’Onofrio et al., 2014).

The present study extends the findings by Rommelse et al. (Rommelse et al., 2008b, Rommelse et al., 2008c, Rommelse et al., 2008a, Rommelse et al., 2007b, Rommelse et al., 2007c, Rommelse et al., 2007a, Rommelse et al., 2008d) by testing whether ADHD-related cognitive deficits are only present in unaffected siblings from MPX ADHD families. . If correct, then the use of cognitive endophenotypes in the search for ADHD risk genes might be of particular use for MPX, but not SPX ADHD. Further, we aimed to examine whether cognitive impairments may be different in SPX and MPX ADHD as indicated by dissimilar cognitive profiles in SPX and MPX ADHD patients. So far, no studies have been undertaken that differentiate between single- and multi-incidence ADHD, but it is plausible that different heritable forms of ADHD might result in dissimilar cognitive disabilities.

Methods and materials

Participants

ADHD families were recruited as part of the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study (as previously described in Rommelse et al., 2008a). Inclusion criteria for all participants were at least two biological siblings (in case families: at least one child with a clinical diagnosis of ADHD) and one biological parent willing to participate, offspring age between 4 and 20 years, European Caucasian descent, an $IQ \geq 70$, and no diagnosis of autism, epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile-X-syndrome. All children and parents were carefully phenotyped for ADHD using validated and standardized questionnaires and diagnostic interviews. Families were stratified into SPX and MPX based on the number of affected individuals. SPX families were required to have a single-affected proband, a minimum of one male sibling and all siblings and parents of the proband unaffected by ADHD; MPX families were required to have two or more affected individuals. A total of 31 ADHD SPX nuclear families (including 31 probands and 47 unaffected siblings), 171 ADHD MPX nuclear families (including 264 probands and 123 unaffected siblings), and 142 control nuclear families (263 children) were included in the current study, see Table 1 for sample characteristics and Supplementary Table 1 for a full description of phenotyping and family classification (available online).

[Insert Table 1 about here]

Measures

Cognitive functioning was examined across a range of domains. Full scale IQ was prorated by four subtests of the Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale: Similarities, Vocabulary, Block Design and Picture Completion (Wechsler, 2002, Wechsler, 2000). The forward condition of Digit Span was used to obtain an indication of verbal attention. Four executive function tasks were included: response inhibition, visual and verbal working memory, and set shifting. Response inhibition was measured with the commonly used Go-NoGo paradigm where participants were instructed to withhold a response when the NoGo target was depicted. Visual and verbal working were measured by instructing the participants to correctly reproduce sequences of figures (visual) or digits (verbal) that increased in difficulty after each succeeded trial. Set shifting was measured by administering a task that required a mixture of compatible and incompatible responses, hypothesized to require a higher level of cognitive flexibility. Motor functioning was measured using a simple reaction time task and a motor control task. Last, a timing measure was included to measure the

variability of motor timing. Table 2 provides an overview of the neurocognitive tasks used. For full task descriptions, see Appendix 1 (available online) or elsewhere (Rommelse et al., 2008a).

[Insert Table 2 about here]

Procedure

Neurocognitive assessment of the children with ADHD and their siblings took place at the VU University Amsterdam or at the Radboud university medical center in Nijmegen, the Netherlands and is described in more detail elsewhere (Rommelse et al., 2008c). Control children were tested in a quiet room at their school. To avoid possible inter-rater or location effects, cognitive performance was measured using standardized computerized tasks with fixed settings and computer-calculated outcome measures (e.g. error percentages or mean reaction times) across the two sites. In addition, all examiners were thoroughly trained using a standardized training protocol and were regularly supervised and observed during task administration to monitor standardized assessment across sites and examiners. Stimulants were discontinued for at least 24 hours before testing and non-stimulants according to their plasma half life to allow for sufficient wash-out. Children were motivated with small breaks and received a gift at the end of the session. Additional data collected included blood or saliva samples and behavioral data of all family members. The study was approved by the local medical ethics board. After the study procedures had been fully explained, parents and children (12 years and older) signed for informed consent. Children younger than 12 years of age were asked to give their assent for participation.

Data-analyses

The percentage of missing data was <5% for all dependent measures, except for stop signal reaction time (SSRT). Here, 8.4% of the data were missing. Missings were imputed by means of Expectation Maximization (Tabachnick and Fidell, 2001). Analyses were carried out with and without expectation maximization, which revealed similar results and led to the same conclusions. Results were therefore reported with missing data replaced. To account for the influence of age and sex on neurocognitive performance, we regressed scores for each measure on age and sex and used the unstandardized residuals as dependent variables. Most of the unstandardized residuals were not normally distributed, therefore, a van der Waerden transformation was applied to normalize the dependent measures (Norusis, 1992). This also facilitated the comparison between variables since variables were all depicted on the same scale. A number of dependent variables were mirrored so that the

z-scores of all measures had the same meaning: lower z-scores indicated poorer performance (e.g. more errors or more variable responses).

Linear mixed models (LMM) were used to account for the dependency in the data due to inclusion of siblings and probands by estimating a random intercept. Dependent variables were the neurocognitive measures and group was the independent variable. We contrasted specific groups of interest to answer our research questions. LMM analyses were run with group defined as (a) probands versus unaffected siblings versus controls, separately for SPX and MPX families, to examine whether cognitive deficits were present in (SPX and MPX) probands and MPX, but not SPX, unaffected siblings, (b) SPX versus MPX unaffected siblings to examine whether cognitive performance of first-degree relatives was poorer in MPX compared to SPX families, and (c) MPX versus SPX probands to examine whether potentially different heritable forms of ADHD would result in (dis)similar cognitive profiles in ADHD patients. Furthermore, within family discrepancy scores (estimated mean of proband minus mean of unaffected sibling) in SPX versus MPX families were compared to examine whether within family contrast was higher in SPX than MPX families. A False Discovery Rate (FDR) correction with a q-value setting of 0.05 was applied to control for multiple testing (Benjamini, 2010). Given the unequal sample size for MPX and SPX families, emphasis was given to effect sizes next to the p-values. Effect sizes (Cohen's d) were calculated to define small ($d = .20$), medium ($d = .50$), and large effects ($d = .80$) (Cohen, 1988). All analyses were carried out in SPSS version 20.

Results

Cognitive measures sensitive for SPX-MPX stratification

Endophenotypes in MPX but not SPX ADHD families

Testing our first hypothesis, we found indeed that SPX unaffected siblings were unimpaired compared to controls on all cognitive domains (all p -values $>.17$, effect sizes in terms of Cohen's d ranging from .00-.22) except for verbal working memory ($p=.029$, $d=.32$), whereas MPX unaffected siblings performed poorer than controls on all cognitive domains (p -values $<.033$, d -values $=.21-.49$), except for baseline variability ($p=.151$, $d=.15$). Moreover, comparisons between SPX and MPX unaffected siblings revealed a significantly better performance of SPX unaffected siblings in three domains, namely visual working memory ($p=.024$, $d=.40$), inhibition ($p=.005$, $d=.51$), and time estimation ($p=.005$, $d=.49$). Within-family discrepancy (proband-unaffected

sibling contrast) was larger for SPX probands than for MPX probands for visual working memory ($t=2.65$, $p=.012$). SPX probands differed significantly from their unaffected siblings on TIQ, visual working memory, and variability of time estimation (p -values $<.009$, d -values $=.53-.74$), whereas MPX probands differed from their unaffected siblings only on TIQ ($p<.001$, $d=.29$), see Figure 1 and Table 3.

[Insert Figure 1 about here]

[Insert Table 3 about here]

Cognitive deficits in MPX versus SPX ADHD probands

Testing our second hypothesis, we found that the cognitive profiles of SPX and MPX probands were highly similar. Both probands from SPX and from MPX families performed significantly worse than controls on estimated TIQ, verbal and visual working memory, and variability of time estimation (SPX: p -values $<.006$, d -values $=.52-.71$; MPX: p -values $<.001$, d -values $=.43-.64$) and could not be dissociated from each other (p -values $>.20$, d -values $<.22$).

Impairments in verbal attention, response inhibition, set shifting, and stability of motor control appeared to be most pronounced in MPX ADHD probands. Relative to normal controls, MPX probands showed significant impairments (p -values $<.029$, d -values $=.21-.45$), whereas SPX probands showed no problems on inhibition ($p=.341$, $d=.18$), set shifting ($p=.218$, $d=.25$), or motor control problems ($p=.445$, $d=.13$). The significant difference between SPX probands and controls on verbal attention ($p=.043$, $d=.38$) did not survive FDR correction (q -value $=.132$). However, SPX and MPX probands could not be dissociated from each other (p -values $>.15$, d -values $<.28$) nor from their unaffected siblings (p -values $>.27$, d -values $<.22$) on these domains and within-family discrepancy did not differ between SPX and MPX families (t -values $<.85$, p -values $>.30$), see Figure 2.

[Insert Figure 2]

Measures not sensitive to SPX-MPX stratification

A few domains were insensitive to SPX-MPX stratification. First, for verbal working memory, a performance intermediate between cases and controls was found in MPX and SPX unaffected siblings. Both SPX and MPX unaffected siblings performed significantly worse than controls ($p=.029$, $d=.32$ and $p=.006$, $d=.27$, respectively),

but similar to their affected brothers/sisters (p -values $>.13$, d -values $<.25$). However, the difference between SPX unaffected siblings and controls became non-significant after FDR correction (q -value $=.077$). Second, probands and unaffected siblings from both SPX and MPX families were equally unimpaired on baseline variability (p -values $>.10$, d -values $<.16$), see Table 3.

Discussion and conclusion

In this study, we aimed to examine whether the cognitive architecture underlying SPX and MPX ADHD families is different and useful for parsing the etiological heterogeneity of ADHD. Based on the assumption that individuals from SPX families are more likely than individuals from MPX families to develop ADHD as a result of sporadic genetic and/or non-genetic causes strictly personal to the patient, we hypothesized that shared cognitive deficits between affected and unaffected siblings are present in MPX, but not SPX families. Further, we hypothesized that potentially different heritable forms of ADHD might result in dissimilar cognitive profiles in SPX and MPX ADHD probands. Consistent with our hypothesis, SPX unaffected siblings were unimpaired compared to controls, except for verbal working memory, whereas MPX unaffected siblings showed an intermediate performance between cases and controls on most domains. Furthermore, the cognitive profiles of SPX and MPX probands were highly similar, except that (a) impairments in verbal attention, response inhibition, set shifting and stability of motor control were more pronounced in MPX probands than in SPX probands, and (b) when compared to their unaffected siblings, impairments in TIQ, visual working memory and timing abilities were more pronounced in SPX cases compared to MPX cases.

Results largely confirmed the hypothesized dissociation between SPX and MPX families based on cognitive performance of probands and their unaffected siblings. Indeed, unaffected siblings from MPX families demonstrated a similar (but milder) cognitive vulnerability profile as probands from those families, whereas unaffected siblings from SPX families were indistinguishable from controls on all measures but verbal working memory. The former finding replicates previous analyses in this sample (Rommelse et al., 2008c, Rommelse et al., 2008a, Rommelse et al., 2008b, Rommelse et al., 2007b, Rommelse et al., 2007a, Rommelse et al., 2007c, Rommelse et al., 2008d) as well as many previous studies without stratification according to family history. The latter finding is novel and indeed suggests that in a percentage of ADHD cases (15.3% in our sample) different modes of inheritance may underlie the disorder in the proband that are mostly not shared with the unaffected family members. These SPX probands further seem to be relatively more strongly impaired in TIQ, visual

working memory and timing abilities. When using unaffected siblings as an ideal reference group (viewed as indexing the ‘full potential’ of children with ADHD had they not developed the disorder), an ‘SPX subtype’ of ADHD may relate to factors that particularly decrease overall intelligence, visual working memory and time estimation. (Rare) genetic variations in genes associated with IQ/intellectual (dis)ability and working memory (e.g. *COMT*) (Green et al., 2013, Boonstra et al., 2008), or environmental factors that have a detrimental effect on the development of the brain (e.g. prematurity, low birth weight and fetal distress) (Bilder et al., 2013), might thus play particularly important roles in the development of SPX ADHD. This suggests that sporadic ADHD might be more prevalent among children with lowered (but still normal) intelligence levels. In contrast, the classical response inhibition difficulties as well as verbal attention and motor coordination problems were less outspoken in SPX versus MPX ADHD. This may suggest that factors related to these traits (e.g. genetic polymorphisms in *DAT1* are associated with response inhibition (Boonstra et al., 2008)) are less involved in SPX forms of ADHD. The dopamine-modulated basal ganglia neurocircuits are proposed to underpin inhibitory control and also play an important role in motor control (Sonuga-Barke, 2005, Fliers et al., 2009). Moreover, dopamine plays an important role in attention and auditory processing (Bailey, 2012). Abnormalities in structure and function of these circuits caused by genetic variation might thus be hypothesized to be less often observed in SPX ADHD. It is challenging to explain why SPX unaffected siblings were impaired in verbal working memory and not other cognitive domains. A possible explanation might be that auditory (or verbal) tasks are generally more difficult than visual tasks, because auditory measures are more closely related to the attentiveness required for daily life than visual measures (Park et al., 2011). Given that (a) SPX unaffected siblings displayed somewhat elevated levels of ADHD traits compared to controls (see sample characteristics) and (b) inattention is a core characteristic of ADHD, this might explain why SPX unaffected siblings did show some problems in this particular area. However, since SPX unaffected siblings were unimpaired on verbal attention, this suggests that the verbal working memory deficit is not fully explained by attention problems. Working memory problems in SPX unaffected siblings did not extend to the visuo-spatial domain. Possibly, verbal working memory is most sensitive to (mild) susceptibility for ADHD. Additional research is however needed to further investigate this issue. In any case, these preliminary findings suggest that the rarer SPX forms of ADHD may have partially different cognitive underpinnings compared to MPX forms of ADHD and a different pattern of familial-determined cognitive vulnerabilities, with minimal cognitive vulnerabilities in unaffected siblings.

In contrast to the situation in SPX families, when selectively analyzing cognitive traits in family members from MPX families, virtually for all cognitive domains a strong endophenotypic group pattern was

found: unaffected siblings originating from families in which at least two members had ADHD, showed substantial cognitive vulnerabilities, similar to their affected sibling. These findings suggest that in families with shared risk factors for ADHD, using cognitive traits to detect these underlying causal factors may be a powerful approach. Particularly impairments in inhibition, motor control, visual working memory and time estimation seem sensitive to such effects and promising areas for further research in this context. These neurocognitive functions may be useful in creating more homogeneous subgroups of patients with (MPX) ADHD. This reduces heterogeneity and may facilitate our understanding of the involved biological processes, boost power for genetic analyses, as well as shed light on the functional outcomes of genes (Gottesman and Gould, 2003).

The direct comparison between SPX and MPX probands revealed very similar cognitive problems. These findings suggest a phenomenon referred to in developmental psychopathology as equifinality (Cicchetti and Rogosch, 1996), that is, even though partly different developmental pathways might underlie SPX and MPX ADHD, these result in quite similar cognitive deficits and also in similar severity of ADHD symptoms. By examining cognitive functions or behavioral symptoms alone, these different underlying etiological factors cannot be identified. Instead, causal effects might be muted by the presence of multiple distinct subgroups of ADHD patients with different etiologies (Nigg et al., 2005). Although the reality of equifinality is well-recognized, few solutions have been provided to tease etiological heterogeneity apart. Stratification into SPX and MPX seems therefore highly relevant in defining relevant subgroups in order to facilitate research that aims to unravel these multiple pathways leading to the same cognitive impairments and ADHD symptomatology

Our findings further highlight the fact that there is clearly no 1:1 relationship between cognitive problems and behavioral problems (de Zeeuw et al., 2008): unaffected siblings from SPX and MPX families did not differ from each other regarding (the absence of) ADHD symptoms, yet substantial cognitive vulnerabilities were only present in MPX unaffected siblings. This corroborates with the conclusions from a systematic review on cognitive (dis)similarities in ADHD persisters and remitters; both were equally impaired at follow-up on almost all domains assessed (van Lieshout et al., 2013). It suggests that cognitive vulnerabilities and behavioral problems are to some extent disentangled during the course of development. It has been hypothesized that neurocognitive deficits in ADHD are epiphenomena instead of core causal factors, that are related to the same etiological factors but do not mediate between genes and behavior (Kendler and Neale, 2010, Kebir and Jooper, 2011, Rommelse et al., 2011). This could explain the highly similar cognitive profiles of MPX affected and unaffected siblings (who are likely to share etiological risk factors for ADHD), and the highly deviant cognitive

profiles of SPX affected and unaffected siblings (where causal risk factors for ADHD appear strictly personal to the patient). More longitudinal studies are definitely needed in this fascinating area of research.

A number of limitations of this study need to be considered. First, only a small proportion of families could be classified SPX. Therefore, we calculated effect sizes to accompany statistical testing. We nonetheless restricted interpretation to significant findings; it follows that our study needs replication in larger samples. Further, boys were overrepresented in both proband groups and in SPX unaffected siblings, but were underrepresented in MPX unaffected siblings and controls. This was due to the fact that a) in childhood, ADHD is more frequently diagnosed in males and b) the presence of male unaffected siblings was only required for SPX, but not MPX families. However, we do not believe that this has affected the results, since the effect of sex was controlled for in this study.

In all, our results support the hypothesis that a partly different cognitive architecture may underlie SPX and MPX forms of ADHD, which becomes evident when contrasting cognitive performances *within* families. When using performance of unaffected siblings as a reference, TIQ, visual working memory and time estimation are particularly impaired in SPX ADHD, suggesting sporadic (non-)genetic causes acting predominantly on these domains. Response inhibition and motor control seem relatively unimpaired in SPX forms of ADHD. In contrast, familial (MPX) ADHD is related to a wide range of cognitive vulnerabilities, translated to comparable (but milder) impairments in unaffected siblings. These findings suggest that different causal pathways may lead up to –on the surface- comparable cognitive deficits and ADHD symptoms in children with ADHD, and that SPX-MPX stratification may be a step forward in unravelling these various causal pathways. Clinically, subgroups of ADHD patients may have distinct prognoses and benefit most from different treatment strategies (Nigg et al., 2005), which indicates that awareness of the impact of family history on the presence of ADHD traits and cognitive impairments in probands and their unaffected siblings is relevant for the development of treatment plans and for genetic counseling.

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Key bullet points

- The aetiology of ADHD is heterogeneous; small disease-increasing effects of multiple common genetic variants likely play a role in multi-incidence (MPX) families, while rare genetic variants (e.g. de novo mutations) likely play a role in single-incidence (SPX) families.
- We may improve our understanding of aetiological heterogeneity of ADHD by studying cognitive deficits in SPX versus MPX ADHD.
- A partly different cognitive architecture appears to underlie SPX and MPX ADHD. MPX ADHD is related to a wide range of cognitive vulnerabilities, translated to comparable (but milder) impairments in unaffected siblings. SPX ADHD is related to TIQ, visual working memory and time estimation impaired in probands but not in siblings.
- Clinically, SPX and MPX ADHD patients may have distinct prognoses and benefit from different treatment strategies.

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TABLE 1. Sample characteristics

	Controls (c)		ADHD probands				Unaffected siblings				Group contrasts ADHD vs. controls
			1. SPX		2. MPX		3. SPX		4. MPX		
	M	sd	M	sd	M	sd	M	sd	M	sd	
Number of kids	N = 263		N = 31		N = 264		N = 47		N = 123		
Age	11.7	3.2	11.8	2.5	11.5	2.6	10.9	3.4	11.6	3.5	ns
Sex (% males)	41.1		87.1		73.6		74.5		38.2		1=2>3>4=c
CPRS DSM-IV Combined Scale	46.5	4.5	76.1	8.0	74.8	10.3	48.1	7.3	50.6	9.5	1=2>3=c, 3=4, 4>c
CTRS DSM-IV Combined scale	46.4	4.5	69.7	8.0	67.7	10.8	48.3	6.3	50.1	7.9	1=2>3=c, 3=4, 4>c

Note. ADHD = attention-deficit/hyperactivity disorder; SPX = simplex; MPX = multiplex, CPRS = Conners Parent Rating Scale; CTRS = Conners teacher rating scale; c = controls; 1 = SPX probands; 2 = MPX probands; 3 = SPX unaffected siblings; 4 = MPX unaffected siblings; ns = non significant

TABLE 2. Description of the neurocognitive tasks

Task	Measurement potential	Dependent variables
Intelligence		
Vocabulary, Similarities, Block Design, Picture Completion ^a	intelligence	Total IQ (TIQ)
Attention		
Digit Span ^a	Verbal attention	maximum span forward
Executive functions		
Stop Task	inhibition	stop signal reaction time (SSRT)
Digit Span ^a	verbal working memory	maximum span backwards
Visuospatial Sequencing	visuospatial working memory	percentage correct identified targets in correct order (part forward)
Shifting Attentional Set Visual	set shifting	percentage errors
Motor functions		
Baseline Speed	baseline variability	variability of reaction time (SD in ms).
Tracking	motor control without continuous adaptation	stability (SD of distances in mm). (non-preferred hand)
Timing		
Motor Timing	timing estimation	variability in reaction time (SD)

Note. For task details, see Appendix 1 or elsewhere (Rommelse et al., 2008a).

^a based on Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale

TABLE 3. Means and standard errors of the transformed task variables for SPX and MPX probands, their unaffected siblings and normal controls

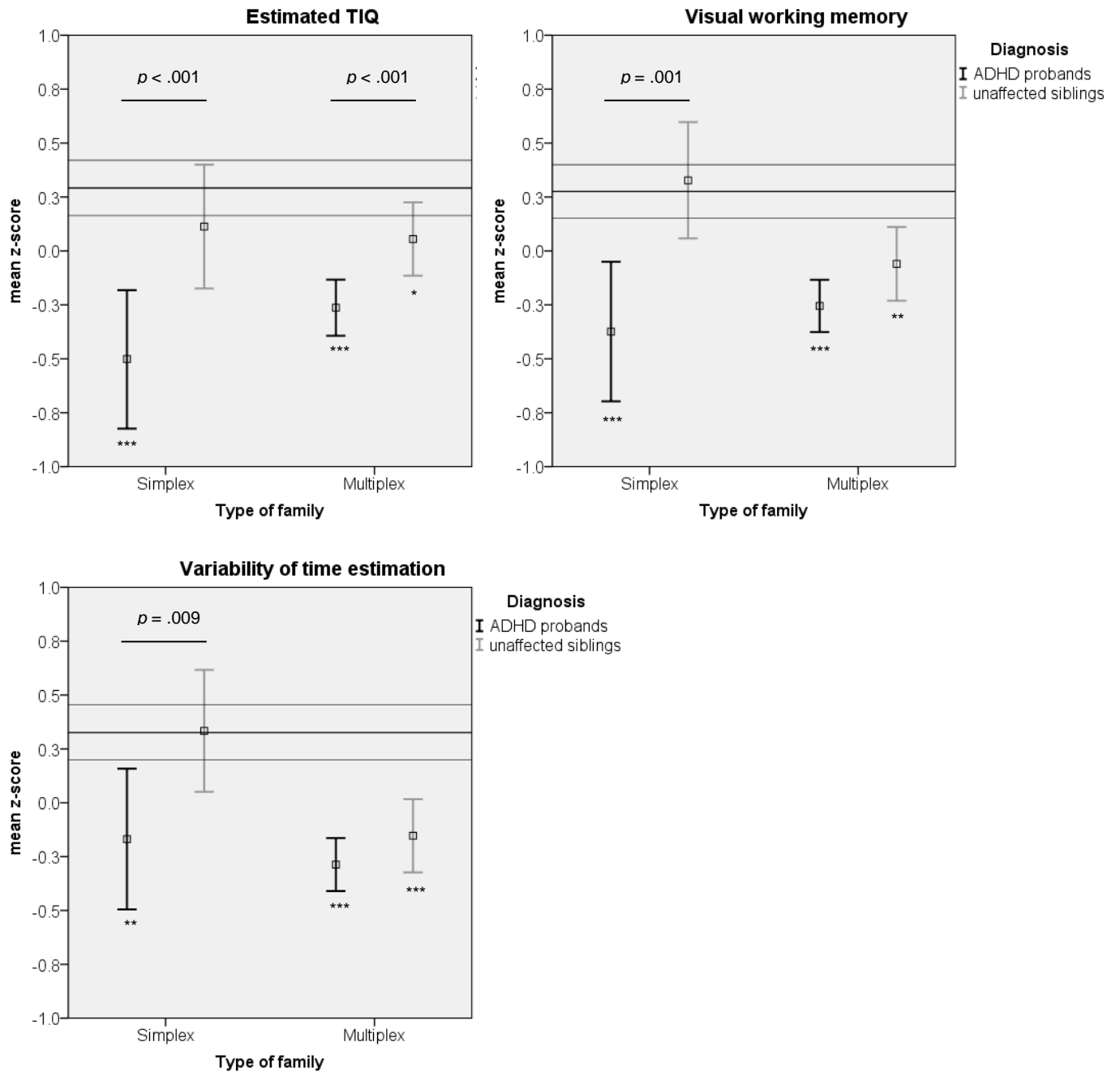
Controls (c)			ADHD probands		unaffected siblings		Group contrasts		Within family contrasts		Comparisons between SPX and MPX family members			
											probands		unaffected siblings	
	M (se)	Family type	M	se	M	se	<i>p-values*</i>	<i>d-values*</i>	<i>t</i>	<i>p</i>	<i>p</i>	<i>d</i>	<i>p</i>	<i>d</i>
<u>Endophenotypes in MPX but not SPX ADHD families</u>														
TIQ	.29 (.07)	SPX	-.50	.17	.11	.15	<.001/.264/<.001	.71/.16/.62	1.421	.164	.212	.22	.496	.05
		MPX	-.26	.07	.06	.09	<.001/.033/<.001	.49/.21/.29						
Visual WM	.28 (.06)	SPX	-.37	.17	.33	.14	<.001/.726/.001	.67/.05/.74	2.652	.012	.502	.11	.024	.40
		MPX	-.26	.06	-.06	.09	<.001/.002/.054	.56/.35/.20						
Variability of time estimation	.33 (.06)	SPX	-.17	.17	.33	.14	.006/.960/.009	.52/.00/.53	1.909	.065	.516	.12	.005	.49
		MPX	-.29	.06	-.15	.09	<.001/<.001/.168	.64/.49/.14						
<u>Cognitive deficits in MPX but not SPX ADHD probands</u>														
Verbal attention	.23 (.06)	SPX	-.14	.17	.04	.14	.043/.239/.378	.38/.20/.19	.463	.677	.880	.03	.463	.12
		MPX	-.17	.06	-.08	.09	<.001/.006/.352	.41/.32/.10						
Inhibition SSRT	.24 (.06)	SPX	.07	.17	.29	.15	.341/.792/.273	.18/.05/.22	.834	.322	.157	.28	.005	.51
		MPX	-.20	.06	-.22	.09	<.001/<.001/.856	.45/.47/.02						
Set shifting % errors	.13 (.06)	SPX	-.11	.18	.06	.15	.218/.690/.444	.25/.07/.17	.651	.518	.853	.04	.248	.17
		MPX	-.07	.06	-.11	.09	.029/.032/.708	.21/.25/.04						
Stability of motor control	.22 (.07)	SPX	.07	.18	-.02	.16	.445/.167/.666	.13/.22/.09	.833	.411	.159	.27	.777	.07
		MPX	-.19	.06	-.09	.09	<.001/.005/.340	.39/.28/.10						
<u>Measures not sensitive to SPX-MPX stratification</u>														
Verbal WM	.26 (.07)	SPX	-.35	.18	-.10	.15	.001/.029/.237	.55/.32/.25	.673	.654	.363	.16	.606	.07
		MPX	-.19	.06	-.03	.09	<.001/.006/.138	.43/.27/.16						
Baseline variability	.08 (.07)	SPX	-.01	.18	-.10	.15	.667/.878/.632	.08/.16/.09	.400	.691	.764	.06	.340	.02
		MPX	-.07	.06	-.08	.09	.108/.151/.898	.14/.15/.01						

Note. ADHD = attention-deficit/hyperactivity disorder, SPX = simplex, MPX = multiplex, M = mean, se = standard error, WM = working memory. SSRT = stop signal reaction time.

Significant group contrasts after FDR correction, are presented in bold.

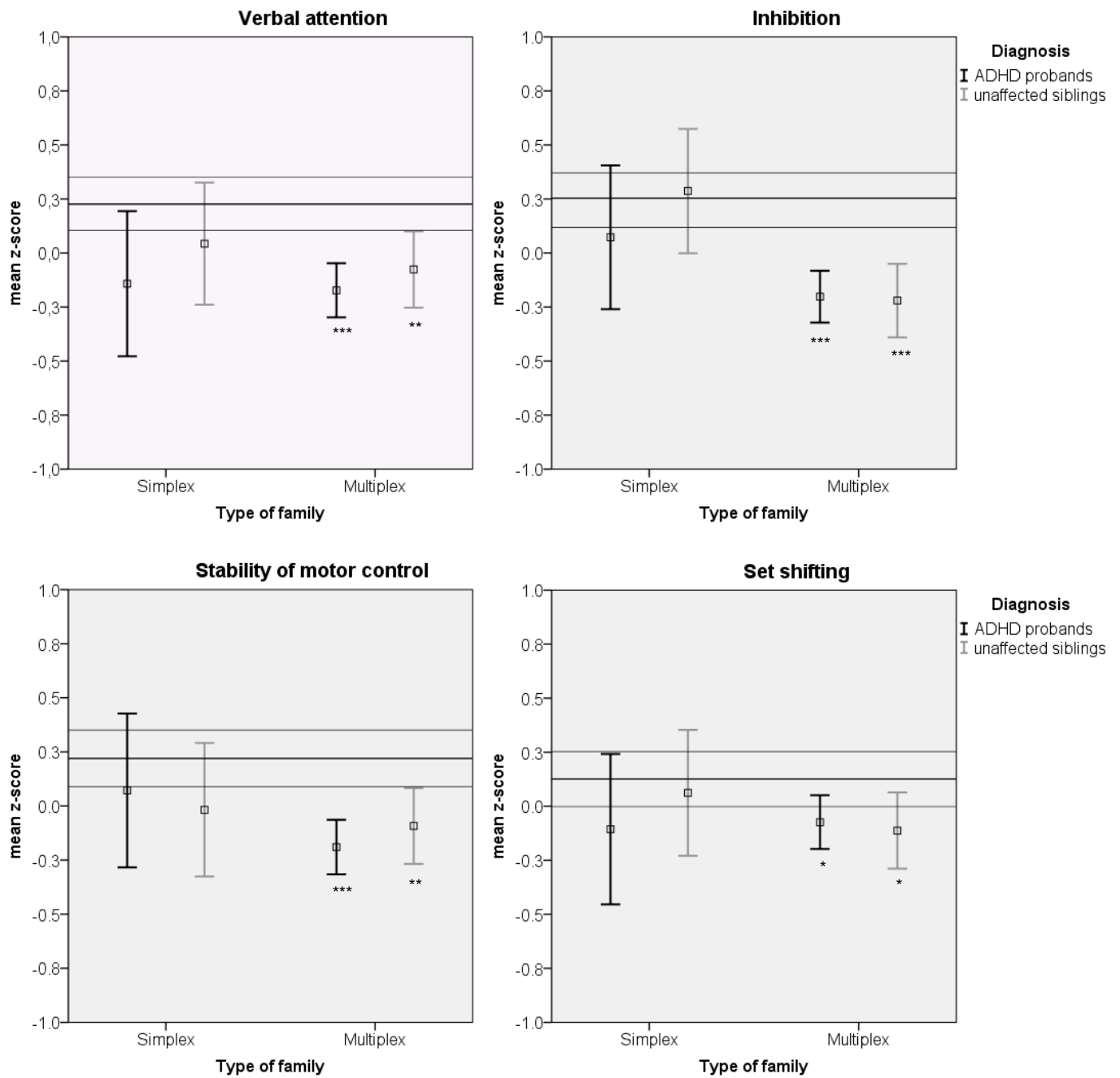
**p*-values and *d*-values are presented in the following order: probands vs. controls / siblings vs. controls / probands vs. siblings

FIGURE 1. Cognitive deficits in MPX, but not SPX unaffected siblings from ADHD families.



Note. The interpolation lines represent the mean z-score and the 95% CI of normal controls. The error bars represent the 95% confidence interval (CI). Lower z-scores indicate worse performance. Significant group differences that survived FDR correction between case groups and controls, are depicted using asterisks (** $p < .001$, ** $p < .01$, * $p < .05$). SPX and MPX ADHD probands performed significantly worse than controls and could not be dissociated from each other on TIQ, visual memory and variability of time estimation. MPX, but not SPX unaffected siblings showed similar cognitive impairments on these domains.

FIGURE 2. Cognitive domains most pronounced in MPX ADHD families



Note. The interpolation lines represent the mean z-score and the 95% CI of normal controls. The *error bars* represent the 95% confidence interval (CI). Lower z-scores indicate worse performance. Significant group differences that survived FDR correction between case groups and controls, are depicted using asterisks (*** $p < .001$, ** $p < .01$, * $p < .05$). Probands and unaffected sibling from MPX, but not SPX ADHD families were impaired on verbal attention, inhibition, set shifting and motor control.